

## REMARKS

### I. Status of the Application

Claims 1-46 are presently pending in the application. Applicants gratefully acknowledge the Examiner's withdrawal of the objection of claim 25 for failing to further limit the subject matter of a previous claim. Applicants also gratefully acknowledge the Examiner's withdrawal of the rejection of claims 6-8, 41, 43, 45 and 46 under 35 U.S.C. § 112, second paragraph, the rejection of claims 1, 3, 4, 6-13, 23, 24, 27, 29, 32, 33, 36-41 under 35 U.S.C. § 102(a) as being anticipated by Carulli et al. (*Journal of Cellular Biochemistry Supplements*, 1998, vol. 30/31, p. 286-296), the rejection of claims 5, 14-22, 25, 26, 28, 30, 31, 34, 35, 42 and 44-46 under 35 U.S.C. § 103(a) as being unpatentable over Carulli et al. in view of Lockhart et al. (WO 97/10365), and the rejection of claims 2 and 42 under 35 U.S.C. § 103(a) as being unpatentable over Carulli et al. in view of Serafini et al. (U.S. Patent No. 6,110,711) and Van Gelder et al. (U.S. Patent No. 6,291,170).

Claims 1-13, 23, 24, 27, 29, 32, 33, 36-41 and 43 stand newly rejected under 35 U.S.C. § 103(a) as being unpatentable over Carulli et al. (*Journal of Cellular Biochemistry Supplements*, 1998, vol. 30/31, p. 286-296) in view of Phillips et al. (*Methods*, 1996, vol. 10, p. 283-288). Claims 14-22, 25, 26, 28, 30, 31, 34, 35, 42 and 44-46 stand newly rejected under 35 U.S.C. § 103(a) as being unpatentable over Carulli et al. in view of Phillips et al. and further in view of Lockhart et al. (WO 97/10365). Applicants respectfully traverse these rejections. A *prima facie* case of obviousness requires three showings:

First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations. The teaching or suggestion to make the claimed combination and the reasonable

expectation of success must both be found in the prior art, and not based on Applicants' disclosure.

Manual of Patent Examining Procedure, 8<sup>th</sup> ed., § 2142. These requirements of a *prima facie* case of obviousness are not met for either of the two rejections under 35 U.S.C. § 103(a).

**II. Claims 1-13, 23, 24, 27, 29, 32, 33, 36-41 and 43 Are Not Obvious over Carulli et al. in View of Phillips et al.**

At page 3, paragraph 5 of the instant Office Action, claims 1-13, 23, 24, 27, 29, 32, 33, 36-41 and 43 stand rejected under 35 U.S.C. §103(a) as being unpatentable over Carulli et al. (*Journal of Cellular Biochemistry Supplements*, 1998, vol. 30/31, p. 286-296) in view of Phillips et al. (*Methods*, 1996, vol. 10, p. 283-288). The Examiner is of the opinion that the claimed invention is *prima facie* obvious in view of the cited references. Applicants respectfully traverse this rejection.

Applicants' claims are directed to novel methods for monitoring multiple gene expression wherein RNA is isolated from a sample comprising fewer than 1000 cells or from a single cell, a first population of cDNA is generated from the RNA, the first population of cDNA is linearly amplified to produce a linearly amplified second population of cDNA, the linearly amplified second population of cDNA is labeled with a detectable label, and an array of probes is contacted with the linearly amplified population of labeled cDNA such that relative hybridization of the probes to the linearly amplified population of labeled cDNA and relative expression of at least two genes can be determined. Applicants' claimed invention has been demonstrated to result in linear amplification of cDNA (the ratios of transcripts between the original cellular RNA and the amplified cDNA is preserved), to exhibit an excellent correlation to actual gene expression in the cell, and to accurately and consistently represent genes having low levels of expression in the

cell (specification page 32, line 31 to page 35, line 2; Tietjen et al. (2003) *Neuron* 38:161, give to Examiner Siew at January 20, 2004 interview).

To establish a *prima facie* case of obviousness, the prior art reference (or references when combined) must teach or suggest all the claim limitations. *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991). Carulli et al. fails to teach or suggest linear amplification of cDNA, as required by independent claims 1, 32, 39, 40 and 43 and their dependent claims. Carulli et al. refers to linear amplification methods disclosed by Lockhart et al. [*Nature Biotech.* (1996) 14:1678] that appear to amplify transcripts uniformly, but the methods of Lockhart et al. are directed to linear amplification of antisense RNA, **not cDNA**. The Examiner admits that Carulli et al. fails to teach or suggest obtaining RNA from a sample comprising fewer than 1000 cells, fewer than 100 cells, or from a single cell as required by the pending claims. Thus Carulli et al. fails to teach or suggest each and every element of the claimed invention.

Phillips et al. fails to cure the deficiencies of Carulli et al. The Examiner is of the opinion that since Phillips et al. discloses a method of detecting gene expression from an RNA population derived from a single cell, it would be *prima facie* obvious to apply the teachings of Carulli et al to monitor the expression of two or more genes from a single cell sample as provided for by Phillips et al, thus arriving at the claimed invention. However, nowhere does Phillips teach or suggest a linearly amplified population of **cDNA**. The method of Phillips et al. uses non-amplified double-stranded cDNA reverse-transcribed from cellular RNA as a template for linear amplification of **antisense RNA** (page 284, right column, last paragraph, to page 286, left column). Therefore, Carulli et al. combined with Phillips et al. do not teach or suggest all the claim limitations.

It is well established that a *prima facie* case of obviousness requires some suggestion or motivation to combine the reference teachings with a reasonable expectation of success. MPEP, 8<sup>th</sup> ed., § 2143. The applied references, Carulli et al. and Phillips et al., do not meet this standard. Carulli et al. teaches away from linear amplification of cDNA, stating “protocols that use PCR amplification of the cDNA have typically resulted in inconsistent amplification of the independent messages in the cell or tissue sample” (page 290, left column, second paragraph). Carulli et al. then refers to linear amplification methods disclosed by Lockhart et al. [*Nature Biotech.* (1996) 14:1678] that appear to amplify transcripts uniformly, but the methods of Lockhart et al. are directed to linear amplification of antisense RNA, **not cDNA**.

Phillips et al. also teaches away from linear amplification of cDNA, stating “single-cell PCR is limited in the type of question that can be addressed,” and pointing out problems with limited RNA sample size, limited primer use and thus difficulty monitoring a multiplicity of genes, *Taq* DNA polymerase errors propagated by amplification, and **non-proportional/nonlinear amplification** (page 283, right column, second paragraph). Phillips et al. then states that the antisense RNA linear amplification method avoids many of the above problems associated with cDNA amplification (Page 283, right column, last paragraph to page 284, left column, first paragraph). Therefore, neither Carulli et al. nor Phillips et al. provide any suggestion or motivation to combine their teachings with a reasonable expectation of success to arrive at the claimed linear amplification of cDNA.

Surprisingly, Applicants have found an effective PCR method for linear amplification of cDNA reverse transcribed from a single cell’s RNA population (specification page 27, lines 8-21). “In this manner, and despite the PCR step, the amplified cDNA maintains an accurate

representation of the different cell RNAs. This cDNA synthesis can be done on single cells or groups of cells.” (page 27, lines 18-21)

A *prima facie* case of obviousness cannot be made because both Carulli et al. and Phillips et al. fail to provide motivation to combine their respective teachings with a reasonable expectation of success, and fail to teach or suggest all of the claim limitations. Accordingly, Applicants respectfully request that the Examiner withdraw the 35 U.S.C. §103(a) rejection and allow claims 1-13, 23, 24, 27, 29, 32, 33, 36-41 and 43.

**III. Claims 14-22, 25, 26, 28, 30, 31, 34, 35, 42 and 44-46 Are Not Obvious over Carulli et al. in View of Phillips et al. and further in View of Lockhart et al.**

At page 7, paragraph 2 of the instant Office Action, claims 14-22, 25, 26, 28, 30, 31, 34, 35, 42 and 44-46 stand rejected under 35 U.S.C. §103(a) as being unpatentable over Carulli et al. in view of Phillips et al. (*Methods*, 1996, vol. 10, p. 283-288) and further in view of Lockhart et al. (WO 97/10365). The Examiner is of the opinion that the claimed invention is *prima facie* obvious over the cited references. Applicants respectfully traverse this rejection.

As discussed in section II above, Carulli et al. and Phillips et al. fail to provide motivation to combine their respective teachings with a reasonable expectation of success, and also fail to teach or suggest all of the claim limitations of independent claims 1, 32, 39, 40 and 43 and their dependent claims, which includes claims 14-22, 25, 26, 28, 30, 31, 34, 35 and 45-46. For the same reasons as stated in section II above, independent claim 42 and its dependent claim 44 are not obvious over Carulli et al. in view of Phillips et al. Lockhart et al. fails to cure the deficiencies of the primary references. Nowhere does Lockhart et al. describe linear amplification of cDNA. Lockhart et al. also teaches away from linear amplification of cDNA by teaching towards linear amplification of RNA as a particularly preferred embodiment (page 29,

last paragraph). Therefore, Lockhart et al. fails to provide motivation to combine teachings with Carulli et al. and Phillips et al. with a reasonable expectation of success to arrive at the claimed linear amplification of cDNA.

A *prima facie* case of obviousness cannot be made, and accordingly, Applicants respectfully request that the Examiner withdraw the 35 U.S.C. §103(a) rejection and allow claims 14-22, 25, 26, 28, 30, 31, 34, 35, 42 and 44-46.

#### IV. Conclusion

Having addressed all outstanding issues, Applicants respectfully request reconsideration and allowance of the case. To the extent the Examiner believes that it would facilitate allowance of the case, the Examiner is requested to telephone the undersigned at the number below.

Respectfully submitted,

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